

## REMARKS/ARGUMENTS

Applicants respectfully request reconsideration and allowance of this application in view of the amendments above and the following comments.

On the question of priority, the Examiner finds claims 31-35, 37 and 38 are only entitled to benefit of the instant filing date of October 15, 2003, and not to the benefit of the filing dates of any of the priority applications. However, Applicants respectfully disagree. Certainly, these claims are supported by Provisional Application Serial No. 60/485,969, filed July 10, 2003, and, therefore, unquestionably entitled to the benefit thereof. These claims are supported by the provisional application as follows:

Claim	Clause	Support in Provisional USSN 60/485,969
31	a	Page 7, next-to-last paragraph
	b	Page 8, second paragraph
	c	Page 6, description of Figure 12
	d	Page 8, second paragraph, very last line
32		Page 8, second paragraph, first line in definition of "A"
33		Page 8, second paragraph, second line in definition of "A"
34		Page 8, second paragraph, first line in definition of "C"
36	a	Page 5, description of Figure 7
	b	Page 5, description of Figure 7
	c	Page 7, third paragraph
	d	Page 5, description of Figure 6
37	a	See above
	b	Examples
	c	Page 5, description of Figure 7; and Examples
38		Examples

In short, Applicants respectfully submit that at least claims 31-34 and 36-38 are entitled to benefit of the filing date of Provisional Application Serial No. 60/485,969, filed July 10, 2003. An early notice to that effect is earnestly solicited.

Claim 26 was objected to because the term “the nonhuman vertebrate” appeared in line 3. In response, Applicants have deleted the objectionable term.

Claims 27 and 38 were rejected under 35 USC § 112, first paragraph, as being broader than the enabling disclosure.

Claims 1, 5, 6, 9-12, 15-17, 20-24, 26, 27, 37 and 38 were rejected under 35 USC § 112, first paragraph, as being broader than the enabling disclosure.

Applicants respond to **both** lack of enablement rejections together. Applicants believe that the problem in each case is that the mice claimed in claims 27 and 38 lack a phenotype. In response, Applicants have amended these two claims to require a specific phenotype in accordance with the Examiner’s stated enabled phenotype in the last paragraph on page 3 of the Office Action. Particularly, the Examiner indicated enablement “wherein expression of said shRNA results in reduced expression of the gene targeted by said shRNA in said mouse.” Thus, Applicants have provided that “said mouse, as a result of expression of the shRNA contained in said expression vector, exhibiting a reduction in the activity of a product of one of its genes targeted by said shRNA compared to a mouse of the same species that does not express said shRNA.”

In view of the foregoing, Applicants believe that both lack of enablement rejections are overcome. An early notice to that effect is earnestly solicited.

Claims 31-35, 37 and 38 were rejected under 35 USC § 103(a) as being obvious over Lowe et al. (“Lowe”), US 2008/0226553, Soriano et al. (“Soriano”), US 6,461,864, and Kunath et al. (“Kunath”), *Nature Biotechnology*, 21: 559-561 (2003). In response,

Applicants respectfully point out that Lowe's effective U.S. filing date is September 27, 2003. In contrast, as noted above, claims 31-34, 37 and 38 clearly are entitled to the benefit of Provisional Application Serial No. 60/485,969, filed July 10, 2003, which is earlier than Lowe's effective U.S. filing date. Consequently, Lowe is not prior art against these claims, and this rejection should be immediately withdrawn as to those claims.

With respect to claim 35, Applicants respectfully point out that this claim is directed to specific sequences, and there is nothing in the cited combination of references that teaches or suggests these specific sequences to persons skilled in the art. Consequently, the cited combination of references could hardly have rendered these specific sequences *prima facie* obvious to persons skilled in the art.

Indeed, Applicants respectfully submit that it was not obvious for a person skilled in the art that a single copy shRNA construct under the control of an RNA polymerase III (pol III) dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II (pol II) dependent locus.

None of the cited references is instructive in respect to the strategy of targeted integration of a shRNA construct under the control of a pol III dependent promoter into a pol II dependent locus to achieve ubiquitous RNA interference in a living organism. Consequently, in the cited combination of references, there is a complete failure of any reasonable teaching or suggestion evidencing that a person skilled in the art had any reasonable expectation of success to express a shRNA with an ubiquitously active Pol III construct integrated into a Pol II locus. In the absence of such teaching or suggestion,

Applicants respectfully submit that the cited combinations of references could have rendered any of the rejected claims *prima facie* obvious to persons skilled in the art.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Claims 1, 5, 6, 9, 10, 15, 16, 20-24, 26, 27 and 30 were rejected under 35 USC § 103(a) as being obvious over McCaffrey et al. (“McCaffrey”), *Nature*, 418: 38-39 (2002) or Beach et al. (“Beach”), US 2003/0084471, and Bronson et al. (“Bronson”), *Proc. Natl. Acad. Sci. USA* 93: 9067-9072 (1996).

Claims 1, 5, 31-34 and 36-38 were rejected under 35 USC § 103(a) as being obvious over McCaffrey or Beach and Bronson and further in view of Soriano.

Claims 11, 12 and 17 were rejected under 35 USC § 103(a) as being obvious over McCaffrey or Beach and Bronson and Soriano and further in view of Ohkawa et al. (“Ohkawa”), *Hum. Gene Ther.*, 11: 577-85 (2000).

Applicants respond to the previous **three** obviousness rejections together, as they are all premised basely on the combination of McCaffrey or Beach and Bronson.

Applicants respectfully submit that it was not obvious for a person skilled in the art that a single copy shRNA construct under the control of an RNA polymerase III (pol III) dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II (pol II) dependent locus.

McCaffrey and Beach describe a method of gene knockdown in a mouse by administering a shRNA expression vector. However, these references are not instructive in respect to the strategy of targeted integration of a shRNA construct under the control of a pol III dependent promoter into a pol II dependent locus to achieve ubiquitous RNA interference in a living organism.

McCaffrey demonstrates transient inhibition of gene expression by injection of purified siRNA or a plasmid encoding a shRNA expression vector into the tail vein of mice. Using this approach, gene knockdown is restricted to liver and persists only a few days. Although the result demonstrates that the mechanism of RNAi mediated gene silencing is functional in mice, the reference not informative in respect to transgenic shRNA expression.

On the other hand, Beach demonstrates that a luciferase specific shRNA under the control of the U6 promoter can mediate widespread gene silencing in cultured cell lines (ambiguously referred as 'in vivo' in this document). Random rather than targeted integration of shRNA expression vectors is applied in all experiments presented and the resulting cell lines were not further analyzed concerning the integration site or the number of shRNA copies integrated into the genome. Usually, random integration of DNA vectors results in a concatameric array of multiple copies, whereas single copy integrations are unusual (Martin & Whitelaw 1996, BioAssays 18, p. 919-923). Therefore, Beach does not teach anything about the requirements of the genomic environment to facilitate transgenic shRNA expression.

The failure by Beach and McCaffrey to provide useful information concerning ubiquitous expression of shRNA transgenes in a multicellular organism is not cured by Bronson. Particularly, Bronson did not provide motivation of targeting a shRNA construct under the control of a pol III dependent promoter into to a pol II dependent locus. Rather, Bronson applied homologous recombination at the HPRT locus to introduce a bcl-2 cDNA under the control of a pol II *but not* a pol III dependent promoter. The expression level of the targeted bcl-2 transgenes appeared to be non-ubiquitous and varied between the two different constructs. Therefore, the data suggest that targeted integration into a ubiquitously active locus (such as hprt) does *not* support ubiquitous expression of a transgene or a shRNA under the control of a pol II dependent promoter. The activity of a shRNA construct under the control of a pol III dependent promoter as demonstrated by the present invention is neither taught nor suggested by the reference.

Consequently, in the cited combination of references, there is a complete failure of any reasonable teaching or suggestion evidencing that a person skilled in the art had any reasonable expectation of success to express a shRNA with an ubiquitously active Pol III construct integrated into a Pol II locus. In the absence of such teaching or suggestion, Applicants respectfully submit that none of the various cited combinations of references could have rendered any of the rejected claims *prima facie* obvious to persons skilled in the art.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw these three rejections. An early notice that these three rejections have been reconsidered and withdrawn is earnestly solicited.

Claims 1, 5, 6, 9-12, 15-17, 20-24, 26, 27, 37 and 38 were provisionally rejected on the ground of non-statutory obviousness-type double patenting as being unpatentable over claims 79-82 of copending application Serial No. 11/571,194 in view of Kunath. In response, Applicants respectfully request that this issue be held in abeyance until allowable subject matter is indicated, at which time Applicants will take appropriate action, for example, file a suitable terminal disclaimer or prove patentable distinctness.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

Applicants also believe that this application is in condition for immediate allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (212) 808-0700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,  
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